REMARKS

In the Final Action dated February 26, 2009, claims 1-8, 13 and 16-83 were pending, of which claims 2-4, 6-8, 16-31 and 34-82 were withdrawn from consideration. Claims 1, 5, 13, 16, 32-33 and 83 were under examination and are rejected. Claims 16, 32, 33, and 83 are objected to because they recite non-elected subject matter in the alternative. Claims 1, 5, 13, 16, 32, 33, and 83 are rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the enablement requirement. Claims 1, 5, 13, 16, 32, 33 and 83 are further rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to satisfy the written description requirement.

In response, Applicants have amended the claims and submit the following remarks, together with a Declaration of Dr. Susanne Pedersen under 37 C.F.R.§1.132 (**Exhibit A** with **Exhibits 1-5**). Applicants respectfully submit that this Response addresses each of the Examiner's rejections, and therefore the present application is in condition for allowance. Favorable consideration of all pending claims is therefore respectfully requested.

Claim Amendments

Claim 1 has been amended to delete the hybridization language. Claim 1 as presently recited is directed to a diagnostic method wherein the level of expression is measured of either a nucleic acid molecule comprising the nucleotide sequence as set forth in SEQ ID NO:7 or a nucleic acid molecule comprising a nucleotide sequence complementary to the sequence as set forth in SEQ ID NO:7.

Similarly, independent claim 16 has been amended to delete the hybridization language.

No new matter is introduced by the foregoing amendments.

Information Disclosure Statement and KIAA1199

In Item 5 of the Office Action (page 2), the Examiner states that the Information Disclosure Statement filed on April 29, 2008 has been considered. While Applicants previously stated that instant SEQ ID NO: 7 "corresponds to KIAA1199", the Examiner was not able to establish a relationship between instant SEQ ID NO: 7 and the sequence referred to by Mack et al. (AB033025, GenBank record). The Examiner has provided a report showing that the Examiner attempted to perform a sequence alignment between instant SEQ ID NO: 7 and AB033025, but "no significant similarity" was found. The Examiner has also referred to the alignment Applicants provided in the previous response, which, according to the Examiner, referred to KIAA1199 as a feature "flanking this part of the subject sequence."

Applicants respectfully submit that SEQ ID NO: 7 matches the portion of the genomic sequence of KIAA1199 (AB033025, GenBank record) between exons 1 and 2 based on the exon/intron designations defined by the NCBI database. Further, Applicants observe that the KIAA1199 sequence utilized by the Examiner in performing her alignment is apparently "KIAA1199 mRNA", i.e., without intron sequences, which explains why the Examiner's search did not reveal any similarity.

Applicants further respectfully submit that the NCBI database only annotated one representative transcript of KIAA1199A. However, the AceView database, which is the NCBI EST/cDNA database, demonstrates that there exist other splice variant forms of the KIAA1199 gene, and one of such splice variant forms includes SEQ ID NO: 7. The Pedersen Declaration

provides a detailed explanation in support of Applicants' position in this regard. See paragraphs 6-8 of the Declaration.

Further, Applicants submit that the observations by the inventors, as described in the present application, were based on the isolation of mRNA from colorectal tissue samples. SEQ ID NO:7 could only have been detected if it was actually part of a transcript, as opposed to functioning only as an intron sequence.

Applicants have provided additional experimental data in support of the position that SEQ ID NO:7 is a part of a transcript of the KIAA1199 gene. Such additional data is discussed in detail in the Pedersen Declaration (see paragraph 9 of the Declaration).

Therefore, Applicants respectfully submit that KIAA1199 is not in the "flanking" region of SEQ ID NO: 7, as the Examiner has alleged. Rather, consistent with Applicants' previous submission, instant SEQ ID NO: 7 corresponds to KIAA1199. In fact, as shown in the Pedersen Declaration, SEQ ID NO: 7 is within an mRNA transcript from the KIAA1199 gene.

Claim Objection

Claims 16, 32, 33, and 83 were objected to because they recite non-elected subject matter in the alternative.

Applicants note that rejoinder of the additional combinations which require SEQ ID NO: 7 will be considered once the claims based on elected SEQ ID NO: 7 are found allowable. Therefore, Applicants wish to maintain the recitation of the additional combinations because, as discussed hereinbelow, the subject matter based on elected SEQ ID NO: 7 is patentable.

35 U.S.C. § 112, First Paragraph (Enablement)

Claims 1, 5, 13, 16, 32, 33, and 83 are rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the enablement requirement.

The Examiner has gone through a lengthy analysis based on the eight factors pronounced in In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988). The Examiner concludes that given the broad scope of the claims, the unpredictability of the relevant art, the large quantity of research required to define the unpredictable variables, the lack of guidance provided in the specification, the absence of a working example and the negative teachings in the prior art, it would require undue experimentation for one of skill in the art to practice the method as broadly written.

Applicants believe that the Examiner's rejection is directed to several elements of the claimed method: the sample source, other SEQ ID NO: 7-related sequences, detection of a protein product, and determination of an increase in expression. Applicants respectfully disagree with the Examiner's rejection and will address each of the elements as follows.

Sample source

The claims currently recite "a blood, serum, stool or gastrointestinal tract sample". The Examiner contends that the specification does not teach overexpression of SEQ ID NO: 7 or any variants or homologs thereof in any other tissue, except colorectal tissue biopsy samples obtained from colonoscopy. See page 6 of the Action.

The Examiner appears to have been unconvinced by the evidence Applicants previously provided in the supporting reference to Walgenbach-Brunagel et al. (2008). The Examiner states that it is not prioro predictable what makers can be detected in the blood and which cannot, and that each of the markers to which Applicants refers is supported by extensive

experimentation and data. The Examiner notes that experimentation and data for fecal, blood, or plasma samples are absent in the present case. See page 16 of the Action.

Applicants respectfully submit that in addition to the CCA2 marker disclosed in Walgenbach-Brunagel et al. (2008), several other biomarkers which were initially detected in tissue samples, such as PSA, CEA and CA19-9, are also expressed in blood. Contrary to the Examiner's contention, Applicants respectfully submit that the notion that detection of a biomarker in tissue samples translates to detectable changes in blood or serum levels is well documented and amply supported by the art, and confirmation thereof would not require undue experimentation.

In support of Applicants' position in this regard, Applicants provide additional specific data showing the upregulation in the level of expression of KIAA1199 in stool and plasma samples. Paragraph 12 and the accompanying exhibits of the Pedersen Declaration describe how these data were obtained. Specifically, the results show that increased levels of KIAA1199 protein in stool samples were detected an indirect ELISA using a monoclonal antibody directed to KIAA1199 protein. Dr. Pedersen stated in the Declaration that increased levels of mRNA of KIAA1199 are believed to have also occurred in the same stool samples. Additionally, the results also show that approximately 30% of adenoma patients exhibited a significant increase in KIAA1199 mRNA in the plasma, while only one out of ten normal patients showed an increase in KIAA1199 mRNA in the plasma.

Although actual analysis of stool and plasma samples is not specifically exemplified in the present application, based on the disclosure of colonoscopy samples provided in the present application, the teachings in the art and the common general knowledge of the skilled person, one would have reasonably expected that gene expression which is altered in colorectal neoplasms

would also be detectable in stools and in blood samples. In support of Applicants' position in this regard, Applicants direct the Examiner's attention to the Pedersen Declaration. As Dr. Pedersen explained (paragraph 13), it is well known that all solid tumors are associated with a certain level of apoptosis. Neoplastic cells that are apoptosed are then shed into the stools, where they are detectable. Further, where shedding of cells into stools occurs, there generally also exists apoptotic leakage into the blood.

In light of the data showing elevated levels of expression of KIAA1199 in stool samples, and the fact that colorectal neoplasms which shed cells into the stool are often also associated with leakage into the blood, Applicants respectfully submit tissue biopsies, stool and peripheral blood are all appropriate biological samples for analysis for practicing the claimed invention. As stated in the Pedersen Declaration (paragraph 14), once an upregulated expression of a biomarker is established based on tissue biopsy sample, the experimentation involved in confirming that elevated expression can also be detected in stool and blood samples would be routine and not excessive.

Other SEQ ID NO:7-related sequences

The Examiner contends that the specification does not provide any evidence that an increased expression of a molecule, which hybridizes to SEQ ID NO: 7 but is not 100% identical to SEQ ID NO: 7, can be used as a marker for colorectal adenoma.

In an effort to advance prosecution and without prejudice, Applicants have amended the claims to delete reference to the detection of molecules which hybridize under high stringency conditions to SEQ ID NO:7-containing nucleic acid molecule. As such, it is believed that this aspect of the enablement rejected is obviated.

Detection of a protein product

The Examiner argues that the specification does not demonstrate the detection of a translation product of SEQ ID NO: 7, nor does it demonstrate that a putative translation product is detectable at a different level that could be used as a marker in the claimed methods. See page 8, bottom, of the Action. Further, relying on Chan, the Examiner contends that it is unpredictable as to whether or not the results pertaining to nucleic acid expression, as presented in the instant specification, would be applicable to methods requiring or encompassing the analysis of a protein samples.

Applicants respectfully submit that the specification clearly asserts that the altered levels of expression of a relevant nucleic acid molecule can be detected at both the mRNA level and the protein level. See page 23, lines 16-30 of the specification. Further, the data provided in **Exhibit 4** of the Pedersen Declaration demonstrate that increased levels of the translation product of a SEQ ID NO:7-containing gene was detectable in stool samples of patients with colorectal adenoma. Moreover, the Examiner's attention is also directed to the data provided in the paper by Sabates-Bellver *et al.* (2007) (Exhibit 4 of Applicants' previous response, and attached hereto again as **Exhibit B**) showing increased KIAA1199 protein levels in tissue sections of colorectal adenomas. Therefore, Applicants respectfully submit that the aspect of the claimed methods relating to analysis of a protein sample is fully enabled.

Determination of an increase in expression

The Examiner has questioned the data provided in the specification. Specifically, the Examiner states that there is no mention in the specification as to the range of observed values, the variation among samples or any formal statistical analysis to determine if the differences observed

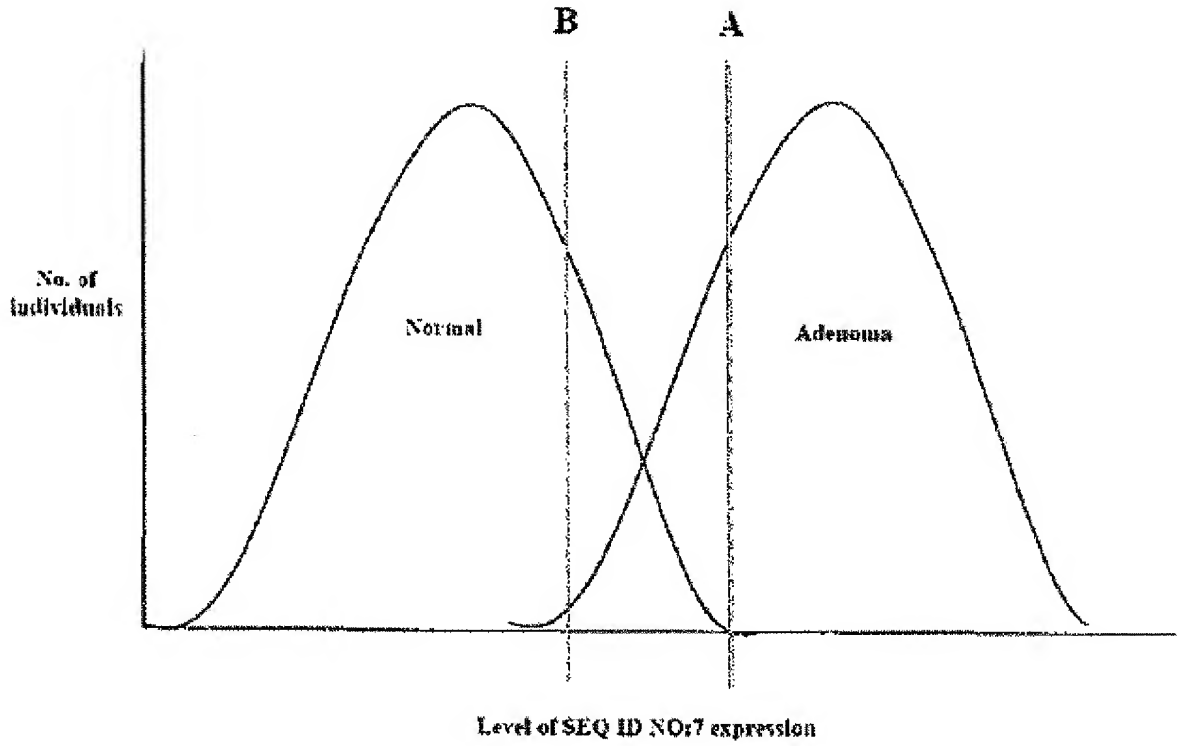
between types can be attributed to sample effects or to the chance of error. The Examiner considers this missing information to be a significant lack of guidance, given that the specification teaches that 19% of normal tissues also over-express both clones related to SEQ ID NO: 7. See page 7, bottom paragraph, of the Action.

Additionally, with respect to the additional supporting data provided in Applicant's previous response, the Examiner is seeking an explanation as to why there appears to exist a different degree of increase in expression of SEQ ID NO: 7 in the new data relative to the original data. Specifically, the new data apparently shows a 4.39-fold increase in SEQ ID NO: 7 while the original data in the specification shows a 45-55 fold increase in expression.

With respect to the guidance and data provided in the specification, Applicants respectfully submit that the invention is based not on any specific fold increase in SEQ ID NO:7 expression, but rather, in the fact that the SEQ ID NO:7 level of expression in adenomas is significantly increased over the level of expression in normal subjects. Importantly, in all adenoma patients, the level of expression of SERQ ID NO:7 is increased over the mean level of expression of SEQ ID NO:7 in normal patients.

It is recognized that there will always occur a few individuals who are at the extremes of a bell curve expression (which is the usual graphical result which one gets when plotting the results of a series of test results for a specific cohort). That is, in the context of SEQ ID NO: 7, there will always occur a few normal individuals who may exhibit a slightly higher level of expression of SEQ ID NO:7 than the majority of individuals appearing in the mean range, while there will always be a few adenoma patients who exhibit a slightly lower level of expression of SEQ ID NO:7 as compared to the mean patient level within an adenoma population. However,

these adenoma patients who exhibit a slightly lower level of expression of SEQ ID NO: 7 are nevertheless exhibiting a higher level of expression than the normal mean value. This can be illustrated by the schematic bell curve graph which appears below. Note that this graph is a schematic depiction only to illustrate the SEQ ID NO: 7 results.



In short, in any diagnostic system, those skilled in the art will be able to make a decision as to where they set their cut-off, above which they regard a patient as falling into the adenoma group and below which they regard the patient as a normal. This cut-off point may vary from one situation to another and a diagnostic clinician must have flexibility to make that decision based on the particular requirements of the situation he or she is dealing with. The two bell curves illustrate

the situation with SEQ ID NO: 7. It is very clear that the bell curve for SEQ ID NO: 7 expression in adenoma is shifted significantly to the right relative to the bell curve for SEQ ID NO:7 expression in normal patients. It is very clear that the mean value for normal patient expression of SEQ ID NO:7 is significantly lower than the mean level of expression of SEQ ID NO:7 for adenoma patients. This is also evident if the mean points in all the other graphs which have been provided to the Examiner are compared.

However, there is a crossover at the upper extreme of the normal bell curve and the lower extreme of the adenoma bell curve. If 100% sensitivity is sought then the cut-off point at which patients are classified as falling into an adenoma group, as opposed to a normal group, would be set at the right hand end of the normal bell curve (point A). This would necessarily, however, result in some patients who have developed adenoma being misclassified as normal's. Accordingly, where there is 100% specificity there would be less sensitivity. However, if one is prepared to reduce specificity, one can increase sensitivity. Accordingly, if the demarcation point for the level of expression of SEQ ID NO: 7 above which adenoma is diagnosed is moved to the left (point B), one can identify with 100% sensitivity patients who have developed adenoma. However, one will also identify a small group of patients who have a slightly higher level of expression of SEQ ID NO: 7 than the normal mean level but who have not developed adenoma. It is a level of this type at which the specificity and sensitivity was set for the experiment described in the specification. The 19% of normal individuals who are found to express a slightly elevated level of SEQ ID NO: 7 were the 19% of individuals who fall within the extreme level of the normal bell curve.

Applicants respectfully submit that the method of the present invention is not meant to

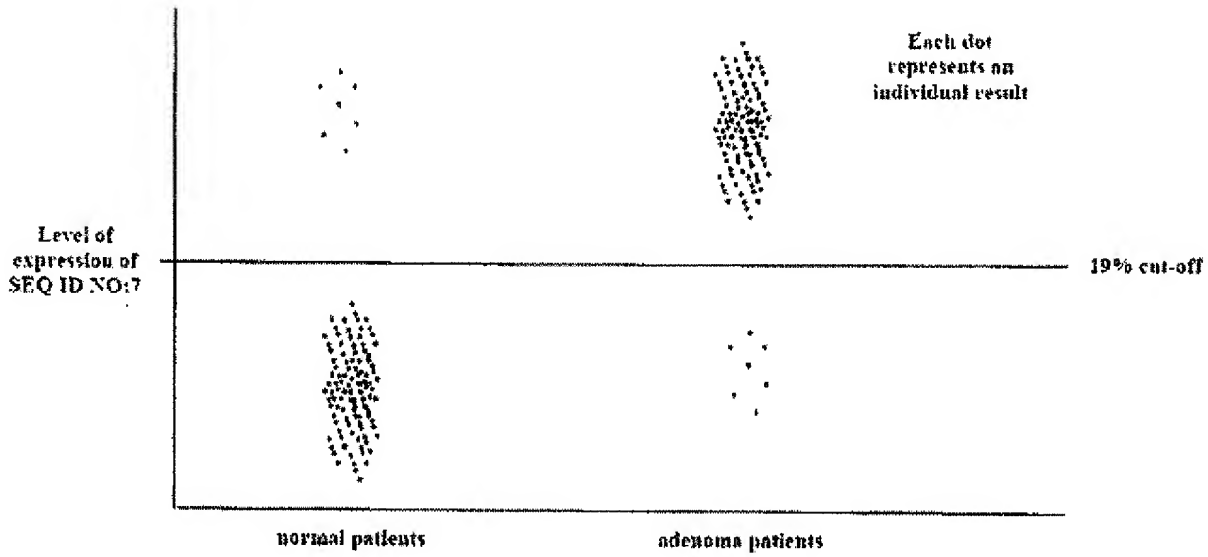
be conclusive but is purported to provide an *indicative* guidance in relation to whether an individual has developed adenoma. Where patients are experiencing a slightly increased level of expression of SEQ ID NO:7, but for example this level of expression is below the mean level of expression which is normally exhibited for adenoma patients, there would be reason to further investigate these patients, such as by virtue of a colonoscopy, to conclusively determine whether or not they have developed adenoma.

One should bear in mind, for example, that in certain situations, such as a large scale government funded screening program, the sensitivity rate is normally set at 6%, rather than 19%, since the government does not want to pay for treatment or further analysis of any patients who may fall within that group of normal patients who express slightly elevated levels of SEQ ID NO: 7. In this situation, the government makes a decision that they are prepared to lose some patients who may have developed adenoma but fall within the lower end of SEQ ID NO:7 expression. This is a further example of the fact that the cut-off rates can be varied as between one situation and another depending on the outcome to be achieved. In terms of the 6% scenario, the nominal cut-off which is depicted as the far left hand cut-off in the above- identified schematic diagram is moved to the right.

It is well within the skills of a person in the art to determine where the cut-off should be set as between the two sets of data. There is no doubt, however, when one examines all the results which have been obtained in SEQ ID NO;7, that there is a statistically significant increase in expression of adenoma patients, when comparing the mean levels of the adenoma patients with the mean levels of normal patients.

A further schematic representation of the situation is depicted below in the form of a

dot point graph, which is also merely a schematic diagram depicting the SEQ ID NO: 7 result trend.



It can be seen that in terms of the normal patients, the bulk of patients exhibit a SEQ ID NO: 7 expression level which falls below the 19% nominal cut-off level while by far the bulk of the adenoma patients express a SEQ ID NO:7 level which falls above that level. There are obviously a handful of patients who fall on the opposite side of the 19% cut-off. However, it does not detract from the significance of these results and their utility in terms of providing an indication as to patients who may have developed adenoma. It is the mean results which one must focus on and not the extreme outliers.

Applicants further respectfully submit that it is not a matter of any difficulty or complexity for the skilled person to obtain a set of results from normal patients and to effectively set up a standard curve along which the nominal cut-off is set. This is routine procedure in the area of clinical diagnostics. Any diagnostic situation will require consideration in relation to issue of

specificity and sensitivity since no biological system is so black and white that all individuals who exhibit a given condition exhibit precisely the same level of fold increase (or decrease) in the expression of a particular molecule. There will always be a range of levels which are observed within a patient population, but provided that the mean level of this range is statistically different to the mean level of the normal range, the molecule is regarded as a useful indicator of the onset of the given disease condition.

As further support, the Pedersen Declaration provided additional data showing that there is a statistically significant increase in SEQ ID NO: 7 levels in adenoma patients as compared to normal patients. See paragraphs 15-17 and Figure 5 in **Exhibit 5** of the Declaration. Referring to paragraph 16 of the Declaration, Dr. Pedersen stated that it is clear from the graphs in Figure 4 and 5 that the mean level of expression of KIAA1199 in normal patients as opposed to adenoma patients is statistically significantly different. There is a statistically significant increase of expression of SEQ ID NO: 7 in each of the graphs. The Examiner should not be focusing on the few patients who appear above and below the mean value, i.e. within the standard deviation, also referred to as the overlapping "whiskers" between the normal and adenoma data sets. This is equivalent to the ends of the bell curves shown in the previously presented bell diagram. It is the middle box which the Examiner should focus on since this represents the data median. The top and bottom of the box are the 25% and 75% interquartile range. The whiskers represent the minimum and maximum observations which are considered outliers. As Dr. Pedersent stated, the phenotypes are differentially expressed by a t-test where $P<0.001$, which represents a statistically significant difference as between the mean of the normal patients as between the 25% and 75% interquartile range and the mean of the adenoma patients.

In terms of the Examiner comments on the different fold changes which are observed in the original discovery data in the specification and the subsequently submitted SEQ ID NO:7 validation data, Applicants again direct the Examiner's attention to the Pedersen Declaration. Dr. Pedersen stated that the fold change differences are due to the fact that the data are generated from fundamentally different technologies which exhibit differences in their limits of detection (paragraph 17 of the Declaration). The data which appeared in the specification as originally filed were derived from gene specific RT-PCR, whereas the data which were submitted together with the last response were generated from whole gene RNA microarrays. Although one uses two entirely different technologies with different limits of detection, the ultimate outcome is consistent. That is, the mean level of expression of SEQ ID NO: 7 is increased in patients who have developed adenoma as opposed to patients who have not. While a variety of screening techniques are suitable for use, whether protein or nucleic acid is being measured, or whether gene specific RNA or whole gene RNA microarrays is used, those skilled in the art would appreciate that the same technique should be used for both normal and test subjects to have a meaningful comparison and diagnosis.

In summation, Applicants have addressed all aspects of the Examiner's enablement rejection. It is respectfully submitted that in light of the guidance provided in the specification, the knowledge of persons of ordinary skill in the art, and the additional supporting data shown in the Pedersen Declaration, those skilled in the art would be able to practice the claimed invention without undue experimentation. Therefore, it is respectfully requested that the Examiner withdraw the enablement rejection.

35 U.S.C. § 112, First Paragraph (Written Description)

Claims 1, 5, 13, 16, 32, 33 and 83 are further rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to satisfy the written description requirement.

The Examiner's rejection is principally directed to the genus of nucleic acid molecules having a nucleotide sequence capable of hybridizing to the complement of SED ID NO: 7 under high stringency conditions.

Applicants believe that the rejection is obviated in light of the amendments which have deleted the hybridization language. Withdrawal of the written description rejection is therefore respectfully requested.

Conclusion

In view of the foregoing amendments and remarks, it is firmly believed that the subject application is in condition for allowance, which action is earnestly solicited

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Encs.: Exhibit A (Declaration and accompanying Exhibits 1-5); Exhibit B.